LAW OFFICES
SUGHRUE, MION, ZINN, MACPEAK & SEAS, PLLC
2100 PENNSYLVANIA AVENUE, N.W.
WASHINGTON, DC 20037-3213
TELEPHONE (202) 293-7060
FACSIMILE (202) 293-7860
www.sughrue.com

January 23, 2001

BOX PCT
Assistant Commissioner for Patents
Washington, D.C. 20231

PCT/JP99/04238
-filed August 5, 1999

Re: Application of Satoshi SASAKI, Yoshihiko SUMI and Reginald Colin HUGHES
PHARMACEUTICAL COMPOSITION HAVING INHIBITORY EFFECT ON OVERPRODUCTION AND ACCUMULATION OF EXTRACELLULAR MATRIX
Our Ref: Q62621

Dear Sir:

The following documents and fees are submitted herewith in connection with the above application for the purpose of entering the National stage under 35 U.S.C. § 371 and in accordance with Chapter II of the Patent Cooperation Treaty:

- ☑ an executed Declaration and Power of Attorney.
- ☑ an English translation of the International Application.
- ☑ six (6) sheet(s) of drawings.
- ☑ International Preliminary Examination Report
- ☑ an English translation of Article 34 amendments (annexes to the IPER).
- ☑ an executed Assignment and PTO 1595 form.
- ☑ International Search Report, Information Disclosure Statement and a Form PTO-1449.

It is assumed that copies of the International Application, and any Articles 19 and 34 amendments as required by § 371(c) will be supplied directly by the International Bureau, but if further copies are needed, the undersigned can easily provide them upon request.

The Government filing fee is calculated as follows:

Total claims	90	-	20	=	70	x	$18.00	=	$1260.00
Independent claims	3	-	3	=		x	$80.00	=	$.00
Base Fee									$860.00
Multiple Dependent Claim Fee									$270.00
TOTAL FILING FEE									$2390.00
Recordation of Assignment									$ 40.00
TOTAL FEE									$2430.00

Checks for the statutory filing fee of $2390.00 and Assignment recordation fee of $40.00 are attached. You are also directed and authorized to charge or credit any difference or overpayment to Deposit Account No. 19-4880. The Commissioner is hereby authorized to charge any fees under 37 C.F.R. §§ 1.16, 1.17 and 1.492 which may be required during the entire pendency of the application to Deposit Account No. 19-4880. A duplicate copy of this transmittal letter is attached.

Priority is claimed from August 6, 1998 based on Japanese Application No. 10-233499 (Pat.Appln.).

Respectfully submitted,

Waddell A. Biggart / Bruce E. Kramer Reg. No. 33,725

Waddell A. Biggart
Registration No. 24,861

WAB/amt

DESCRIPTION

PHARMACEUTICAL COMPOSITION HAVING INHIBITORY EFFECT ON OVERPRODUCTION AND ACCUMULATION OF EXTRACELLULAR MATRIX

FIELD OF INVENTION

The present invention relates to a preventive or therapeutic pharmaceutical composition having an inhibitory effect on the overproduction and the accumulation of extracellular matrix, said composition comprising as an active ingredient a compound that inhibits the biological activity of galectin-3.

BACKGROUND ART

Galectin-3 is a protein that has a molecular weight of about 30 Kd belonging to the family of β-galactoside-binding protein and is a lectin that widely occurs on the cell surface, the cytoplasm and the nucleus of the tissue (see, for example, Barondes, S. H. et al., J. Biol. Chem. (1994) 296: 20807-20810, Hughes, R. C. Glycobiology (1994) 4: 5-12, Wang, L. et al., Biochem. Biophys. Res. Commun. (1995) 217: 292-303, and the like). It is known that galectin-3 binds to a suitable sugar chain portion of glycoprotein present on the cell surface or in the extracellular matrix (ECM) and thereby activates inflammatory cells such as neutrophils, basophils, or macrophages to promote the production of cytokines from these cells (see, for example, Sato, S. et al., J. Biol. Chem. (1994a) 269: 4424-4430, Liu, F. T. Immunol. Today (1993) 14: 486-490), or to suppress the apoptotic death of T cells by its overexpression (see Yang, R-Y, H. et al., Proc. Natl. Acad. Sci. U.S.A. (1996) 93: 6737-6742), and it is believed to be an important protein responsible for inflammatory and immunological reactions.

Furthermore, galectin-3 is also known to play an important role in the formation and repair of the tissue since it is highly expressed during the damage repair period in the rat lung-injured model induced by X-ray irradiation (see Kasper, M. et al., J. Pathol. (1996)

179: 309-316) and it is possibly playing an important role in the formation of kidney tissue in humans during the embryonic stage (see Winyard, P. J. D. et al., J. Am. Soc. Nephrol. (1997) 8: 1647-1657).

The overproduction and the accumulation of extracellular matrix (ECM) such as collagen is believed to be an important factor for the pathogenesis of the fibrosis of tissues such as liver, kidney, lung, heart, pancreas, artery, gastrointestinal tract, thyroid, salivary gland, and skin (see Okada, H. et al., Kidney Int. (1996) 49: Supple. 54: S-37-S-38, Coker, R, K. et al., Eur. Respir. J. (1998) 11(6) : 1218-1221 and the like). ECM is also involved in the maintenance of homeostasis of cellular functions together with the support of parenchymal cells at the physiological conditions. When minor injuries are inflicted to tissues, the repair of the injured tissues is completed by the treatment of the injured tissues by phagocytic cells in the process of inflammatory and repair reactions, the subsequent regeneration of parenchymal cells, and the reconstruction of the supportive substrate, ECM. However, when the injuries are severe or persist for a long time, the overproduction and the accumulation of ECM will cause severe damages in the functions of each tissue. In the liver, for example, lymphocytes and macrophages infiltrate to the periphery of the injured liver cells, and these cellular infiltrates and Kupfer cells or vascular endothelial cells and the like produce cytokines such as PDGF, TGF-β, etc., which then activate Ito cells, a kind of ECM-producing cells. The activated Ito cells proliferate and produce ECM in an excess amount in the Disse's space thereby to cause hepatic fibrosis or hepatic cirrhosis that are said to be a terminal status of the hepatic diseases. In the kidney glomeruli, for example, cytokines such as PDGF and TGF-β are produced from the

cells that have infiltrated into the periphery of the injured kidney cells or endothelial cells in the glomeruli etc. to activate the mesangium cells that are a kind of ECM-producing cells. The activated mesangium cells proliferate while themselves also producing cytokines such as PDGF and TGF-β together with an excessive amount of ECM, creating factors that cause various glomerular diseases, for example, chronic glomerular nephritis, including IgA nephropathy, diabetic nephropathy, glomerular sclerosis and the like. In the interstitial tissue of the kidney also, due to the activation of myofibroblasts, a kind of ECM-producing cells, and the epithelial cells of urinary tubules, these cells excessively produce ECM in the interstitial region of the urinary tubules and form fibrosis of the tubulo-interstitium thereby significantly reducing the renal function. Thus, the ECMs that were overproduced and accumulated in each tissue physically constrain the cellular functions of each cell and substitute for the functional unit of each tissue to cause severe functional disorders of each organ.

For the above-mentioned diseases, adrenal cortical steroids, immunosuppressive agents, anti-platelet agents, anti-coagulants, anti-fibrinolytic agents, ACE inhibitors, and the like are currently used, but no drugs exhibit satisfactory efficacy on the overproduction and the accumulation of ECM, and there is a strong need for agents that have a novel mechanism of action.

Although galectin-3 has been highly expressed at the injured site of the tissue in the rat lung-injured models induced by X-ray irradiation, a model of pulmonary fibrosis, it has not been elucidated whether it can regulate the overproduction and the accumulation of ECM such as collagen and the survival of the mesangium cells that are a kind of ECM-producing cells. Accordingly, it is not known whether the inhibition of the action of galectin-3 can inhibit the overproduction and the

accumulation of ECM and thereby it has a therapeutic and/or preventive usefulness for glomerular nephritis, diabetic nephropathy or tissue fibrosis.

DISCLOSURE OF INVENTION

It is an object of the present invention to provide a pharmaceutical composition having an inhibitory effect on the overproduction and the accumulation of ECM, said composition comprising as an active ingredient a compound that inhibits the biological activity of galectin-3. Furthermore, it is an object of the present invention to provide a therapeutic or preventive agent comprising said compound inhibiting the biological activity of galectin-3 and a pharmaceutically acceptable carrier. In particular, it is an object of the present invention to provide a therapeutic or preventive agent based on a novel mechanism of action of inhibiting the biological activity of galectin-3 for the diseases caused by the overproduction and the accumulation of ECM such as glomerular nephritis, diabetic nephropathy or tissue fibrosis for which no conventional drugs show satisfactory inhibitory effects.

Considering the state of art of the conventional technology, the present inventors have carried out intensive study and have found that galectin-3 is a molecule that can regulate the overproduction and the accumulation of ECM such as collagen and a molecule that can regulate the survival of the mesangium cells which is a kind of ECM-producing cells, and also have found that substances that inhibit the biological activity of galectin-3 can regulate the overproduction and the accumulation of ECM such as collagen, and have thereby completed the present invention.

Thus, the present invention provides a therapeutic or preventive pharmaceutical composition having an inhibitory effect on the overproduction and the accumulation of ECM, said composition comprising as an active ingredient a compound that inhibits the biological

activity of galectin-3, and a pharmaceutically acceptable carrier.

This indicates that compounds that inhibit the biological activity of galectin-3 can be a therapeutic or preventive agent of glomerular nephritis, diabetic nephropathy or tissue fibrosis of which cause is the overproduction and the accumulation of extracellular matrix.

BRIEF EXPLANATION OF THE DRAWINGS

Figure 1 shows a variation in the expression of galectin-3 in the anti-Thy-1.1 antibody-induced rat nephritis model.

A: the kidney of the rats who received no anti-Thy-1.1 antibody, B: day 8 after the administration of anti-Thy-1.1 antibody, C and D: day 14 after the administration of anti-Thy-1.1 antibody.

The asterisk in the figure shows a representative macula densa region, m a representative mesangium region, c a representative crescent body-forming region, the closed arrow tail a representative distal urinary tubule, the closed arrow a representative proximal urinary interstitial tubule, and the open arrow a representative infiltrated macrophage or fibroblast.

Figure 2 shows a variation in the expression of galectin-3 in the UUO rat model.

A: contralateral kidney, B: obstructed kidney

The asterisk in the figure shows a representative macula densa region, the closed arrow tail a representative distal urinary tubule, and the open arrow a representative infiltrated macrophage or fibroblast.

Figure 3 shows the activity of galectin-3 to inhibit the cellular death of the mesangium cells.

Figure 4 shows the activity of galectin-3 to promote the production of collagen type IV by the rat mesangium cells.

Figure 5 shows the suppression by galectin-3 binding-inhibiting glycoprotein of the activity of

galectin-3 to promote the production of collagen type IV production by the rat mesangium cells.

Figure 6 shows the suppression by galectin-3 binding-inhibiting sugar of the activity of galectin-3 to promote the production of collagen type IV production by the rat mesangium cells.

DETAILED DESCRIPTION

Compounds that inhibit the biological activity of galectin-3 for use in the present invention include, for example, the following:

(1) Anti-galectin 3 antibody: mouse anti-galectin 3 monoclonal antibody (for example, an antibody described in Lui, F. T., Et al., J. Biol. Chem. (1996) 35: 6073-6079);

(2) Inhibitors of galectin-3 binding: sugars to which galectin-3 can bind such as Galβ1-4Glc, Galβ1-4GlcNAc, Fucα1-2Galβ1-4Glc, Galα1-3Galβ1-4GlcNAc, Galβ1-3GlcNAcβ1-3Galβ1-4Glc, Galβ1-4GlcNAcβ1-3Galβ1-4Glc, Fucα1-2Galβ1-3GlcNAcβ1-3Galβ1-4Glc, Galβ1-3(Fucα1-4)GlcNAcβ1-3Galβ1-4Glc, Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4Glc, Galβ1-3GlcNAcβ1-3Galβ1-4(Fucα1-3)Glc, Fucα1-2(GlcNAcα1-3)Galβ1-3GlcNAcβ1-3Galβ1-4Glc, NeuNAcα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc, NeuNAcα2-6Galβ1-4GlcNAcβ1-3Galβ1-4Glc, Galβ1-3(NeuNAcα2-6)GlcNAcβ1-3Galβ1-4Glc, Galβ1-3GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc, Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc, Galβ1-3GlcNAcβ1-3Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4Glc, Galβ1-4GlcNAcβ1-6(Galβ1-3GlcNAcβ1-3)Galβ1-4Glc, Galβ1-4GlcNAcβ1-6(Galβ1-4GlcNAcβ1-3)Galβ1-4Glc, Galβ1-4GlcNAcβ1-6(Galβ1-4GlcNAcβ1-2)Manα1-6(Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAc, Galβ1-4GlcNAcβ1-2Manα1-6(Galβ1-4GlcNAcβ1-4(Galβ1-4GlcNAcβ1-2)Manα1-3)Manβ1-4GlcNAc, GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc, Galα1-3Galβ1-4GlcNAcβ1-

3Galβ1-4Glc, GalNAcα1-3(Fucα1-2)Galβ1-3GlcNAcβ1-3Galβ1-4Glc, Galα1-3(Fucα1-2)Galβ1-3GlcNAcβ1-3Galβ1-4Glc, Galβ1-4GlcNAcβ1-6(Galβ1-4GlcNAcβ1-3)Galβ1-4GlcNAcβ1-3Galβ1-4Glc, Galα1-3Galβ1-4GlcNAcβ1-6(Galα1-3Galβ1-4GlcNAcβ1-3)Galβ1-4GlcNAcβ1-3Galβ1-4Glc, Galβ1-4GlcNAcβ1-6(Galβ1-4GlcNAcβ1-3)Galβ1-4GlcNAcβ1-6(Galβ1-4GlcNAcβ1-3)Galβ1-4GlcNAcβ1-3Galβ1-4Glc, Galα1-3Galβ1-4GlcNAcβ1-6(Galα1-3Galβ1-4GlcNAcβ1-3)Galβ1-4GlcNAcβ1-6(Galα1-3Galβ1-4GlcNAcβ1-3)Galβ1-4GlcNAcβ1-3Galβ1-4Glc, Galβ1-4GlcNAcβ1-2Manα1-6(Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAc, Galβ1-4GlcNAcβ1-2Manα1-6(Galβ1-4GlcNAcβ1-4(Galβ1-4GlcNAcβ1-2)Manα1-3)Manβ1-4GlcNAc, Galβ1-4GlcNAcβ1-6(Galβ1-4GlcNAcβ1-2)Manα1-6(Galβ1-4GlcNAcβ1-4(Galβ1-4GlcNAcβ1-2)Manα1-3)Manβ1-4GlcNAc, and blood type B-like sugar chains, or glycolipids comprising the above sugars, or glycoproteins having on the cell surface sugar chains to which galectin-3 can bind, or fragments thereof such as fetuin, asialofetuin, transferrin, asialotransferrin, α1-acid glycoprotein, asialo α1-acid glycoprotein, laminin, fibronectin, CD11b, Lamp-1, Lamp-2, Mac-3, CD98, neutrophil 115 kD protein, neutrophil 180 kD protein (NCA-160/CD66), high-affinity IgE receptor, Fc ε R1, and the like (see, for example, Feizi, T. et al., Biochemistry (1994) 33: 6342-6349, Sato, S., et al., J. Biol. Chem. (1992) 267: 6983-6990). Compounds or antibodies that inhibit the binding of galectin-3 and a sugar chain to which galectin-3 can bind;

(3) Compounds that inhibit the incorporation of galectin-3 into the cell: those which inhibit the biological activity of galectin-3 by acting on galectin-3 receptor or the cells that contain galectin-3 receptor, including, for example, antagonists of galectin-3 receptor, or anti-galectin 3 receptor antibody, AGE or AGE receptor or fragments thereof (see Vlassara, H. et

al., Molecular Medicine (1995) 1: 634-646, and the like);

(4) Compounds that inhibit the transfer of galectin-3 into the cell: inhibitors of galectin-3 transporter protein;

(5) Compounds that inhibit the biological activity of galectin-3 in the nucleus or in the cytoplasm: galectin-3 binding proteins that bind to galectin-3 in the nucleus or in the cytoplasm, or derivatives of nucleic acid or fragments thereof, or compounds that inhibit their binding; and

(6) Compounds that inhibit the expression or secretion of galectin-3: antisense of the galectin-3 gene, compounds that inhibit the function of the promoter region of the galectin-3 gene, compounds that inhibit the transfer of proteins in the cell such as brefeldin A.

Compounds that inhibit the biological activity of galectin-3 for use in the present invention can be formulated to make pharmaceutical compositions having an inhibitory effect on the overproduction and the accumulation of ECM by blending said compounds as active ingredients and pharmaceutically acceptable carriers. The pharmaceutical composition may be therapeutic or preventive agents comprising said compounds and pharmaceutically acceptable carriers.

Diseases caused by the overproduction and the accumulation of ECM include glomerular nephritis, diabetic nephropathy or tissue fibrosis and they also include glomerular nephritis, diabetic nephropathy or tissue fibrosis that are derived from the abnormal proliferation of the mesangium cells.

As used herein, pharmaceutically acceptable carriers can include those that are identical with the excipients mentioned below. The amounts blended of a compound that inhibits the biological activity of galectin-3 and a carrier, without any limitation, follow the dosage of the active ingredient mentioned below, and can be widely selected. The amount of a compound that inhibits the

biological activity of galectin-3 is usually 1 to 70 percent by weight and preferably 5 to 50 percent by weight in the total composition.

The composition thus obtained can be provided as an oral preparation such as a soft capsule, a hard capsule, a tablet, granules, powders, a suspension, a liquid, a syrup etc., an injection, a suppository, or an external preparation using a suitable excipient in a known method.

Such excipients include, for example, plant oils (for example, corn oil, cotton seed oil, coconut oil, almond oil, peanut oil, olive oil, and the like), oily esters such as glyceride oils of middle chain fatty acids, mineral oil, glycerin esters such as tricaprylin and triacetin, alcohols such as ethanol, physiological saline, propylene glycol, polyethylene glycol, vaseline, animal fats, cellulose derivatives (crystalline cellulose, hydroxypropyl cellulose, hydroxypropyl methyl cellulose, methyl cellulose), polyvinylpyrrolidone, cyclodextrin, dextrin, lactose, mannitol, sorbitol, starch and the like.

The dosage of the active ingredient, though depends on the degree of the disease and the age of the patient etc., is about 0.01 mg to 1000 mg per day per capita, preferably 1 mg to 200 mg per day per capita. It is desired that the formulations satisfy such conditions.

EXAMPLES

The present invention will now be explained with reference to the following examples, but the present invention is not limited to these examples in any way.

Example 1. Variation in the expression of galectin-3 in the rat nephritis model induced by anti-Thy-1.1 antibody

Rabbit anti-Thy-1.1 antiserum was obtained by immunizing rabbits subcutaneously on the back with Thy-1.1 antigen purified from rat thymus cells. The rat nephritis model induced by anti-Thy-1.1 antibody was prepared by intravenously administering to the tails of

Sprague-Dawley rats 0.25 ml of the above rabbit anti-Thy-1.1 antiserum diluted 2-fold in phosphate buffered saline together with 0.25 ml of normal rabbit complement (Sigma) according to the method of Okuda et al. (Okuda S., et al., J. Clin. Invest. (1990) 86: 453-462). The rats were sacrificed on day 3, 7, and 14 after the administration of the antibody, and the kidney was extracted from each rat after perfusion with phosphate buffered saline. The extracted kidney was fixed in 4 % (w/v) phosphate buffered formalin, embedded in paraffin, and tissue sections for immunostaining were prepared. The immunostaining of the tissue sections was carried out by using affinity-purified rabbit anti-galectin-3 antibody as the primary antibody, peroxidase-labeled goat anti-rabbit antibody (Sigma) as the secondary antibody, and DAB (Sigma: DAB Tablet) as the chromogenic substrate.

The result of immunostaining of the tissue sections is shown in Figure 1. It was confirmed that in the rats who received no anti-Thy-1.1 antibody, a small quantity of galectin-3 was present in the distal urinary tubule and the macula densa region of the glomerulus, while in the rats who received anti-Thy-1.1 antibody, a large quantity of galectin-3 was observed in the distal and the proximal urinary tubule and the glomerulus on day 8 and 14 after the antibody administration. It was also confirmed that on day 14, infiltrated macrophage and fibroblasts were coexistent with galectin-3 in the pre-fibrotic region of the tubulo-interstitium. This finding confirmed that the expression of galectin-3 is increased at the time of pathogenesis in the rat nephritis models induced by anti-Thy-1.1 antibody, and since galectin-3 was coexistent with infiltrated macrophages or fibroblasts which are a kind of ECM-producing cells and are believed to induce the overproduction of ECM in the pre-fibrotic region, it was strongly suggested that galectin-3 is involved in the formation of fibrosis.

Example 2. Variation in galectin-3 expression in the unilateral ureteral obstruction (UUO)-treated rat interstitial fibrosis model

Female Sprague-Dawley rats (around 8 weeks old at the start of the experiment) were used. Under anesthesia with pentobarbital, complete ureteral obstruction of the left kidney was produced by ligating the ureter with 4-0 silk suture at two points and cutting between the ligatures. The left kidneys from each rat with UUO were harvested as obstructed kidneys and the right kidney as contralateral kidneys after perfused with phosphate buffered saline under diethyl ether anesthesia after 10 days from the operation. Harvested kidneys were fixed with 4 % (w/v) phosphate buffered paraformaldehyde for overnight and transferred to 70% ethanol. Fixed kidneys were embedded with paraffin and sectioned for immunostaining. The immunostaining of the tissue sections was carried out by using an affinity-purified rabbit anti-galectin-3 antibody as the primary antibody, a peroxidase-labeled goat anti-rabbit antibody (Sigma) as the secondary antibody, and DAB (Sigma: DAB Tablet) as the chromogenic substrate.

The result of immunostaining of the tissue sections is shown in Figure 2. The UUO model in which the overproduction and the accumulation of ECM such as collagen is observed in the interstitial region of the urinary tubule is a well known model that induces interstitial fibrosis (see Wright, E. J., et al., Lab. Invest. (1996) 74: 528-537, Yamate, J., et al., Toxicol. Pathol. (1998) 26: 793-801 and the like). In the contralateral kidneys, a small quantity of galectin-3 is present in the distal urinary tubule and the macula densa region of the glomerulus, while in the obstructed kidneys, infiltrated macrophage and fibroblasts were coexistent with galectin-3 in the fibrotic region of the tubulo-interstitium. This finding strongly suggested that galectin-3 is involved in the formation of fibrosis

because galectin-3 was coexistent with infiltrated macrophage or fibroblasts which are a kind of ECM-producing cells and are believed to induce the overproduction of ECM in the fibrotic region.

Example 3. Suppressive activity of galectin-3 on the cellular death of rat mesangium cells

Rat mesangium cells were separated from Sprague-Dawley rats according to the method of Striker et al. (Striker, G. E., et al., Lab. Invest. (1985) 53; 123-128). The separated rat mesangium cells were cultured at 37°C in the presence of 5% CO_2 in the wells of a 96-well plate using an essential medium (DMEM/F12 (1:1) culture medium containing 60 μg/ml penicillin, 100 μg/ml streptomycin, manufactured by Gibco BRL) supplemented with 10% fetal bovine serum. After culturing to semi-confluence, the cultured rat mesangium cells were washed with the essential medium, cultured for 2 days in the essential medium supplemented with 0.1% bovine serum albumin (Sigma), and then were further cultured for 1 to 4 days in the essential medium supplemented with 0.1% bovine serum albumin (Sigma) containing 0 or 50 μg/ml galectin-3 and 0 or 0.4 ng/ml TGF-β. On day 1, 2, 3, and 4 after the addition of galectin-3 and TGF-β, the amount of the cultured rat mesangium cells that survived in their respective wells were measured using as an index the conversion from MTS tetrazolium (Cell Titer 96 Aqueous one solution manufactured by Promega) to formazan by the living cells.

The result is shown in Figure 3. In both of the presence and the absence of TGF-β, galectin-3 was confirmed to suppress the cellular death of the rat mesangium cells, a kind of ECM-producing cells.

Example 4. Promoting effect of galectin-3 on the collagen type IV production by mesangium cells

Rat mesangium cells were separated in a similar

manner to that of Example 3. The separated mesangium cells were cultured at 37°C in the presence of 5% CO_2 in the wells of a 96-well plate using an essential medium (DMEM/F12 (1:1) culture medium containing 60 μg/ml penicillin, 100 μg/ml streptomycin, manufactured by Gibco BRL) supplemented with 10% fetal bovine serum. After culturing to confluence, the cultured rat mesangium cells were washed with the essential medium, cultured for 1 to 2 days in the essential medium supplemented with 0.1% bovine serum albumin (Sigma), and then were further cultured for 3 days in the essential medium supplemented with 0.1% bovine serum albumin (Sigma) containing 0, 10 or 30 μg/ml galectin-3 and 0, 0.1, 0.4 or 1.6 ng/ml TGF-β. On day 3 after the addition of galectin-3 and TGF-β, the amount of type IV collagen accumulated in the culture liquid was measured using a sandwich ELISA method that employed a goat anti-type IV collagen antibody as the immobilized antibody and a biotin-labeled goat anti-type IV collagen antibody (Chemicon) as the primary antibody. The amount of type IV collagen in the culture liquid of each well was normalized by dividing it by the amount of the living cells in each well determined in a similar manner to that described in Example 3.

The result is shown in Figure 4. It was confirmed that galectin-3 promotes the production and/or the accumulation of type IV collagen which is a kind of ECM, from the rat mesangium cells which is a kind of ECM-producing cells, in a similar manner to and in an additive manner with TGF-β.

Example 5. Inhibition of promotion by galectin-3 on collagen type IV production by rat mesangium cells, using a glycoprotein that inhibits galectin-3-binding

Rat mesangium cells were separated in a similar manner to that of Example 2. The separated rat mesangium cells were cultured at 37°C in the presence of 5% CO_2 in

wells of a 96-well plate using an essential medium (DMEM/F12 (1:1) culture medium containing 60 μg/ml penicillin, 100 μg/ml streptomycin, manufactured by Gibco BRL) supplemented with 10% fetal bovine serum. After culturing to confluence, the cultured rat mesangium cells were washed with the essential medium, cultured for 1 to 2 days in the essential medium supplemented with 0.1% bovine serum albumin (Sigma), and then were further cultured for 4 days in the essential medium supplemented with 0.1% bovine serum albumin (Sigma) containing 10 μg/ml of galectin-3 and 0, 0.1, 0.2, 0.5 or 1.5 mg/ml fetuin glycoprotein which is a substance known to inhibit galectin-3 binding (see, for example, Sato, S. et al., J. Biol. Chem. (1992) 267: 6983-6990). On day 4 after the addition of galectin-3 and fetuin, the amount of type IV collagen accumulated in the culture liquid of each well was measured using a sandwich ELISA method that employed a goat anti-type IV collagen antibody (Chemicon) as the immobilized antibody and a biotin-labeled goat anti-type IV collagen antibody (Chemicon) as the primary antibody. The amount of type IV collagen in the culture liquid of each well was normalized by dividing it by the amount of the living cells in each well determined in a similar manner to that described in Example 3.

The result is shown in Figure 5. It was confirmed that a high molecular weight glycoprotein that inhibits galectin-3 binding suppresses the promotion of the production and/or the accumulation of type IV collagen which is a kind of ECM, from the rat mesangium cells which are a kind of ECM-producing cells, by the addition of galectin-3.

Example 6. Inhibition, by galectin-3-binding inhibiting sugar, of the effect of galectin-3 on the promotion of collagen type IV production by rat mesangium cells

Rat mesangium cells were separated in a similar

manner to that described in Example 3. The separated rat mesangium cells were cultured at 37°C in the presence of 5% CO_2 in the wells of a 96-well plate using an essential medium (DMEM/F12 (1:1) culture medium containing 60 μg/ml penicillin, 100 μg/ml streptomycin, manufactured by Gibco BRL) supplemented with 10% fetal bovine serum. After culturing to confluence, the cultured rat mesangium cells were washed in the essential medium, cultured for 1 to 2 days with the essential medium supplemented with 0.1% bovine serum albumin (Sigma), and then were further cultured for 2 days in the essential medium supplemented with 0.1% bovine serum albumin (Sigma) containing 0.4 μg/ml of galectin-3 and 0, 0.25, 0.5, 1 or 2 mM of lacto-n-fucopentaose I which is, a substance known to inhibit galectin-3 binding (LNFP-1, see, for example, Sato, S. et al., J. Biol. Chem. (1992) 267: 6983-6990). On day 2 after the addition of galectin-3 and LNFP-I, the amount of type IV collagen accumulated in the culture liquid of each well was measured using a sandwich ELISA method that employed a goat anti-type IV collagen antibody (Chemicon) as an immobilized antibody and a biotin-labeled goat anti-type IV collagen antibody (Chemicon) as a primary antibody. The amount of type IV collagen in the culture medium of each well was normalized by dividing it by the amount of the living cells in each well determined in a similar manner to that described in Example 4.

The result is shown in Figure 6. It was confirmed that a low molecular weight sugar that inhibits galectin-3 binding suppresses the promotion of the production and/or the accumulation of type IV collagen which is a kind of ECM, from the rat mesangium cells which are a kind of ECM-producing cells, by the addition of galectin-3.

As hereinabove described, it was shown that galectin-3 exhibits an increased expression during pathogenesis in the anti-Thy-1.1 antibody-induced rat

nephritis model, an animal model of mesangial proliferative glomerulonephritis, (Example 1), and thus it was suggested that galectin-3 is involved in the pathogenesis of mesangial proliferative glomerulonephritis. It was also demonstrated that in the anti-Thy-1.1 antibody-induced rat nephritis model and in the obstructed kidneys of the UUO rat model, galectin-3 is coexistent with infiltrated macrophage and fibroblasts in the pre-fibrotic or fibrotic region of the tublo-interstitium (Examples 1 and 2). This finding strongly suggested that galectin-3 is involved in the formation of fibrosis because galectin-3 was coexistent with infiltrated macrophage and/or fibroblasts, a kind of ECM-producing cells, that are believed to induce the overproduction of ECM in the pre-fibrotic or fibrotic region. It was also demonstrated that galectin-3 inhibits the cellular death of the mesangium cells, a kind of ECM-producing cells (Example 3), and that it promotes the production and/or the accumulation of ECM from the ECM-producing cells (Example 4). It was further shown that a substance that inhibits the biological activity of galectin-3 suppresses the promotion of the production and/or the accumulation of ECM from ECM-producing cells by galectin-3 (Examples 5 and 6).

INDUSTRIAL APPLICABILITY

A pharmaceutical composition of the present invention comprising a compound that controls the actions of galectin-3 as active ingredient can be clinically applicable as a therapeutic or preventive agent for glomerular nephritis, diabetic nephropathy or tissue fibrosis.

CLAIMS

1. A pharmaceutical composition having an inhibitory effect on the overproduction and the accumulation of extracellular matrix, said composition comprising as an active ingredient a compound having an inhibitory effect on the biological activity of galectin-3.

2. The pharmaceutical composition according to claim 1, wherein the biological activity of galectin-3 is to promote the production of extracellular matrix from an extracellular matrix-producing cell.

3. The pharmaceutical composition according to claim 1 which exhibits an inhibitory effect on glomerular nephritis, diabetic nephropathy or tissue fibrosis of which cause is the overproduction and the accumulation of extracellular matrix.

4. The pharmaceutical composition according to claim 3, wherein the biological activity of galectin-3 is to promote the production of extracellular matrix from an extracellular matrix-producing cell.

5. The pharmaceutical composition according to any of claims 1 to 4, wherein the compound having an inhibitory effect on the biological activity of galectin-3 is an anti-galectin 3 antibody.

6. The pharmaceutical composition according to any of claims 1 to 4, wherein the compound having an inhibitory effect on the biological activity of galectin-3 is an inhibitor of galectin 3 binding.

7. The pharmaceutical composition according to any of claims 1 to 4, wherein the compound having an inhibitory effect on the biological activity of galectin-3 is a compound that inhibits the incorporation of galectin 3 into the cell.

8. The pharmaceutical composition according to any of claims 1 to 4, wherein the compound that inhibits the biological activity of galectin-3 is a compound that modulates the transfer of galectin 3 into the nucleus.

9. The pharmaceutical composition according to any of claims 1 to 4, wherein the compound that inhibits the biological activity of galectin-3 is a compound that inhibits the physiological activity of galectin 3 in the nucleus or the cytoplasm.

10. The pharmaceutical composition according to any of claims 1 to 4, wherein the compound that inhibits the biological activity of galectin-3 is a compound that modulates the expression or secretion of galectin 3.

11. The pharmaceutical composition according to any one of claims 1 to 10, which is a therapeutic or preventive agent.

12. The pharmaceutical composition according to any of claims 3 to 11, wherein the glomerular nephritis, diabetic nephropathy or tissue fibrosis is glomerular nephritis, diabetic nephropathy or tissue fibrosis, respectively, caused by the abnormal proliferation of mesangium cells.

13. The use of a compound having an inhibitory effect on the biological activity of galectin-3, for the production of a pharmaceutical composition for inhibition of the overproduction and the accumulation of extracellular matrix.

14. The use according to claim 13, wherein the biological activity of galectin-3 is to promote the production of extracellular matrix from an extracellular matrix-producing cell.

15. The use according to claim 13, for treatment of glomerular nephritis, diabetic nephropathy or tissue fibrosis of which cause is the overproduction and the accumulation of extracellular matrix.

16. The use according to claim 15, wherein the biological activity of galectin-3 is to promote the production of extracellular matrix from an extracellular matrix-producing cell.

17. The use according to any of claims 13 to 16, wherein the compound having an inhibitory effect on the

biological activity of galectin-3 is an anti-galectin 3 antibody.

18. (Amended) The use according to any of claims 13 to 16, wherein the compound having an inhibitory effect on the biological activity of galectin-3 is an inhibitor of galectin 3 binding.

19. (Amended) The use according to any of claims 13 to 16, wherein the compound having an inhibitory effect on the biological activity of galectin-3 is a compound that inhibits the incorporation of galectin 3 into the cell.

20. (Amended) The use according to any of claims 13 to 16, wherein the compound that inhibits the biological activity of galectin-3 is a compound that modulates the transfer of galectin 3 into the nucleus.

21. (Amended) The use according to any of claims 13 to 16, wherein the compound that inhibits the biological activity of galectin-3 is a compound that inhibits the physiological activity of galectin 3 in the nucleus or the cytoplasm.

22. (Amended) The use according to any of claims 13 to 16, wherein the compound that inhibits the biological activity of galectin-3 is a compound that modulates the expression or secretion of galectin 3.

23. The use according to any one of claims 13 to 22, which is for a therapeutic or preventive use.

24. The use according to any of claims 15 to 23, wherein the glomerular nephritis, diabetic nephropathy or tissue fibrosis is glomerular nephritis, diabetic nephropathy or tissue fibrosis, respectively, caused by the abnormal proliferation of mesangium cells.

25. A method for inhibition of the overproduction and the accumulation of extracellular matrix, said method comprising administrating a compound having an inhibitory effect on the biological activity of galectin-3, to a subject which needs said inhibition.

26. The method according to claim 25, wherein the

biological activity of galectin-3 is to promote the production of extracellular matrix from an extracellular matrix-producing cell.

27. The method composition according to claim 25 for inhibition of glomerular nephritis, diabetic nephropathy or tissue fibrosis of which cause is the overproduction and the accumulation of extracellular matrix.

28. The method according to claim 27 wherein the biological activity of galectin-3 is to promote the production of extracellular matrix from an extracellular matrix-producing cell.

29. The method according to any of claims 25 to 28, wherein the compound having an inhibitory effect on the biological activity of galectin-3 is an anti-galectin 3 antibody.

30. The method according to any of claims 25 to 28, wherein the compound having an inhibitory effect on the biological activity of galectin-3 is an inhibitor of galectin 3 binding.

31. The method according to any of claims 25 to 28, wherein the compound having an inhibitory effect on the biological activity of galectin-3 is a compound that inhibits the incorporation of galectin 3 into the cell.

32. The method according to any of claims 25 to 28, wherein the compound that inhibits the biological activity of galectin-3 is a compound that modulates the transfer of galectin 3 into the nucleus.

33. The method according to any of claims 25 to 28, wherein the compound that inhibits the biological activity of galectin-3 is a compound that inhibits the physiological activity of galectin 3 in the nucleus or the cytoplasm.

34. The method according to any of claims 25 to 28, wherein the compound that inhibits the biological activity of galectin-3 is a compound that modulates the expression or secretion of galectin 3.

35. The method according to any one of claims 25 to 34, which is for therapeutic or preventive treatment.

36. The method according to any of claims 27 to 35, wherein the glomerular nephritis, diabetic nephropathy or tissue fibrosis is glomerular nephritis, diabetic nephropathy or tissue fibrosis, respectively, caused by the abnormal proliferation of mesangium cells.

ABSTRACT

A pharmaceutical composition having an inhibitory effect on the overproduction and the accumulation of extracellular matrix, said composition comprising as an active ingredient a compound that inhibits the biological activity of galectin-3, which pharmaceutical composition can serve as a therapeutic or preventive agent for glomerular nephritis, diabetic nephropathy or tissue fibrosis, as well as the use of said compound for the production of pharmaceuticals for the above-mentioned use, and a method for inhibition of the overproduction and accumulation of the extracellular matrix.

09/744328

1/6

Fig.1

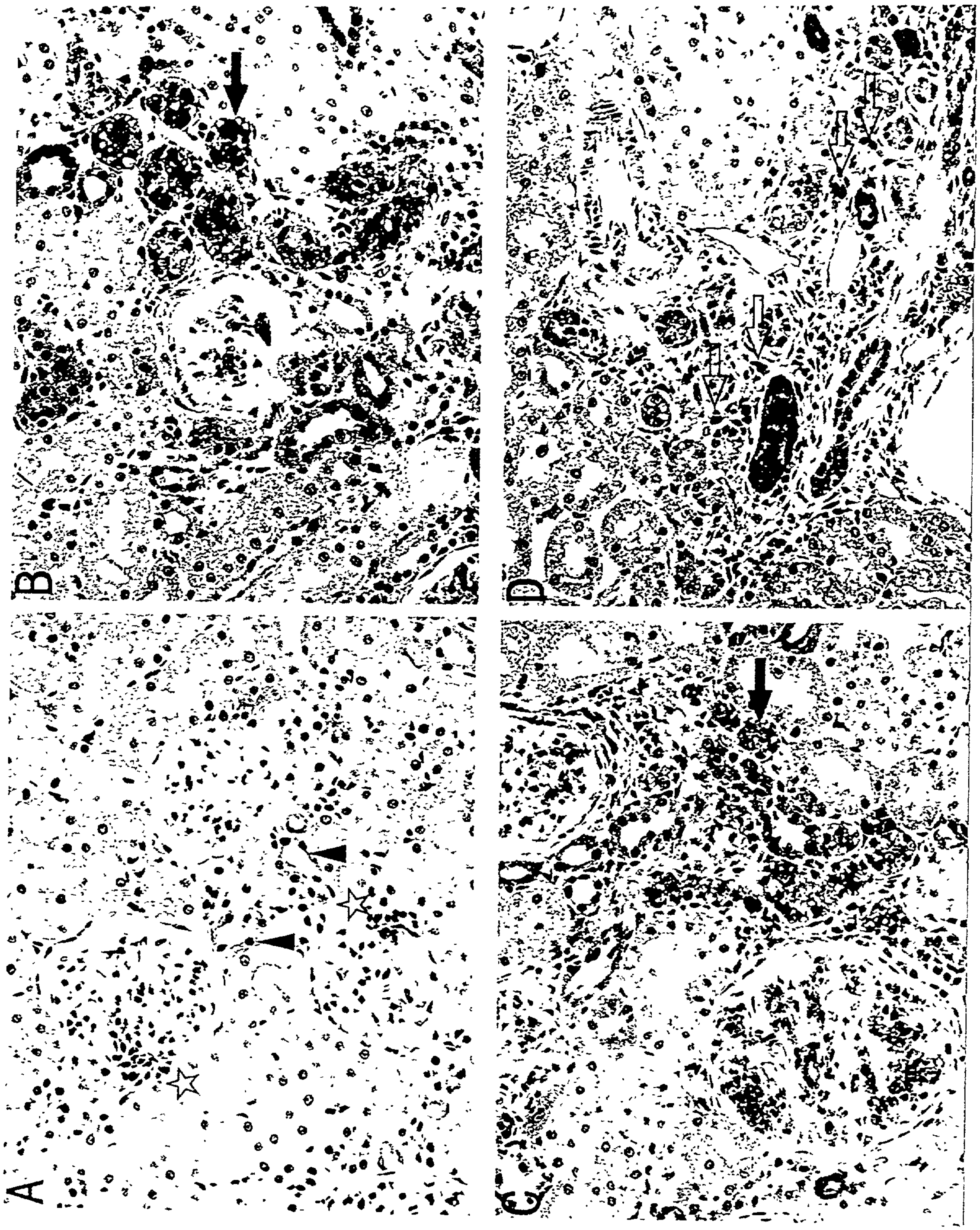
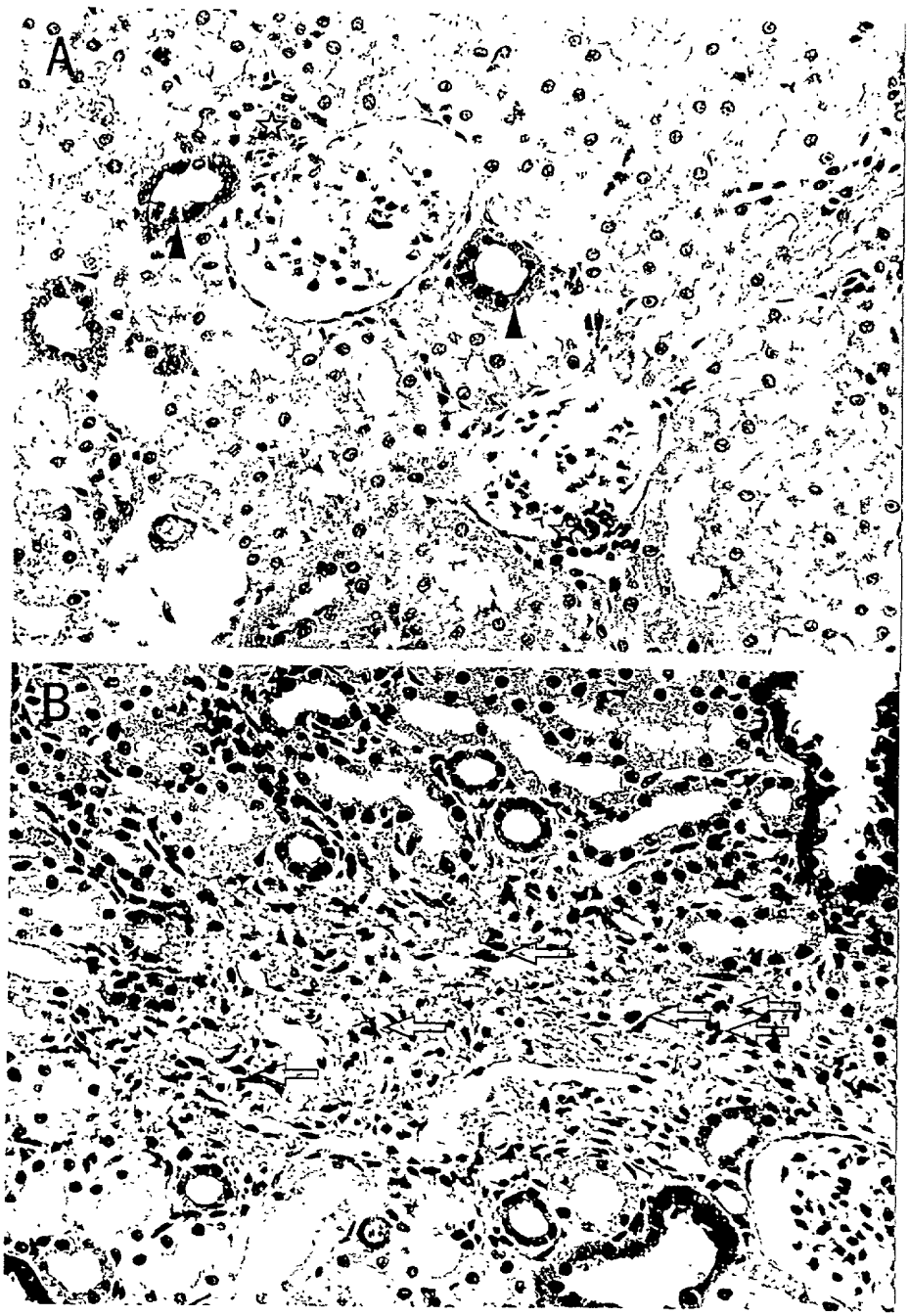
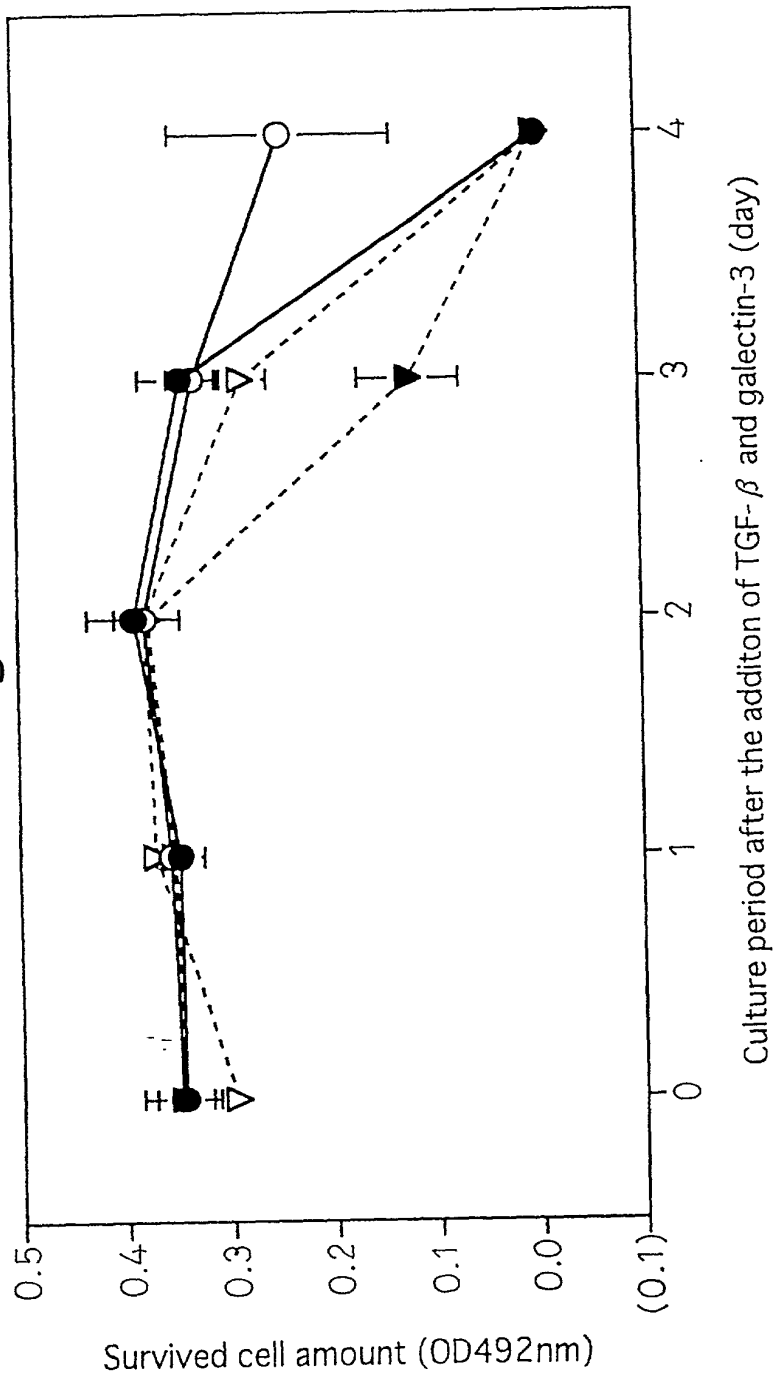


Fig. 2



09/744528

Fig.3



09/744328

4/6

Fig. 4

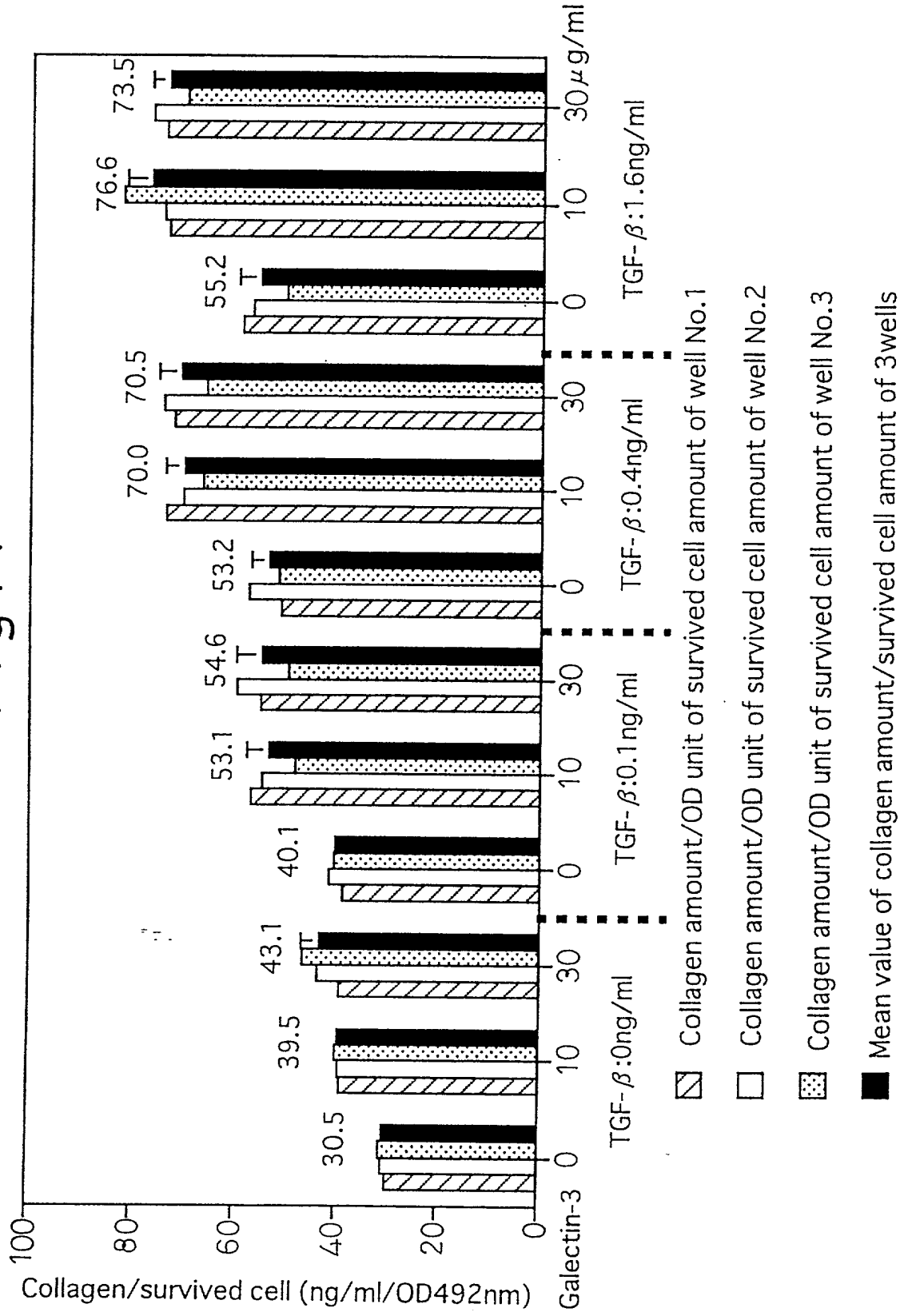


Fig.5

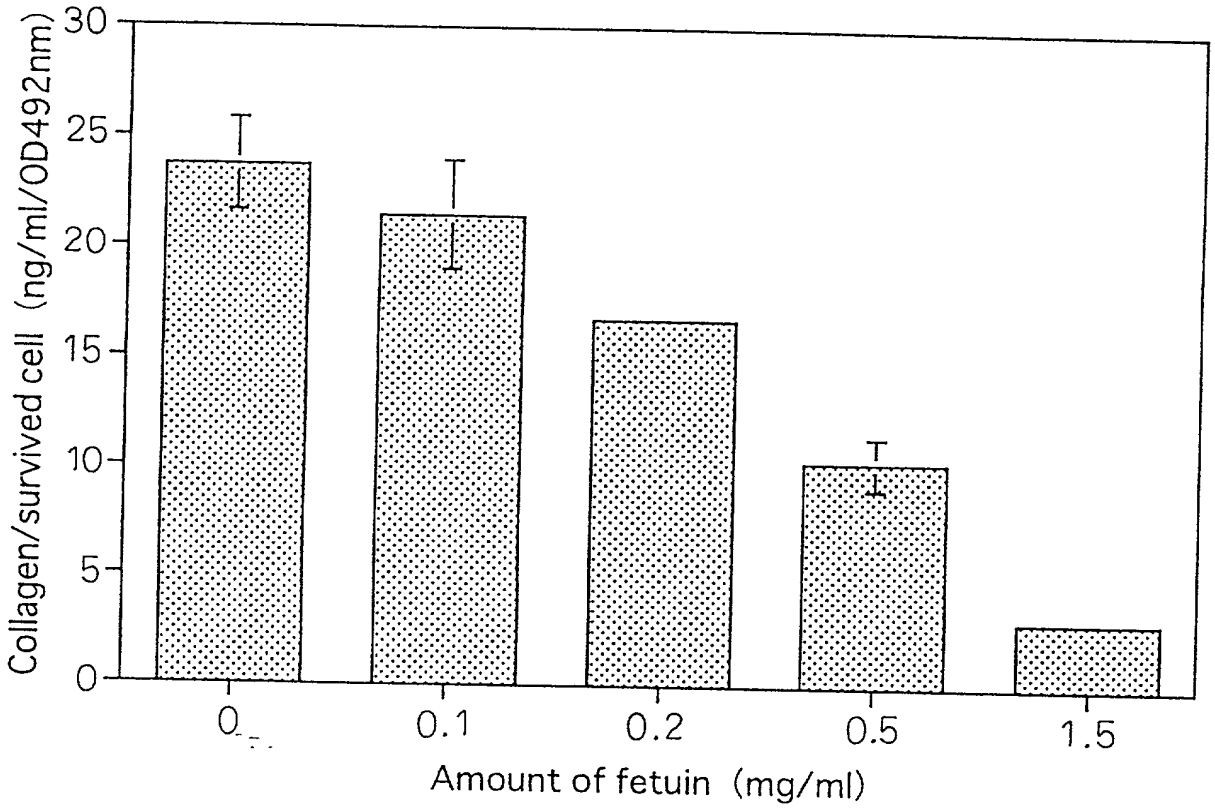
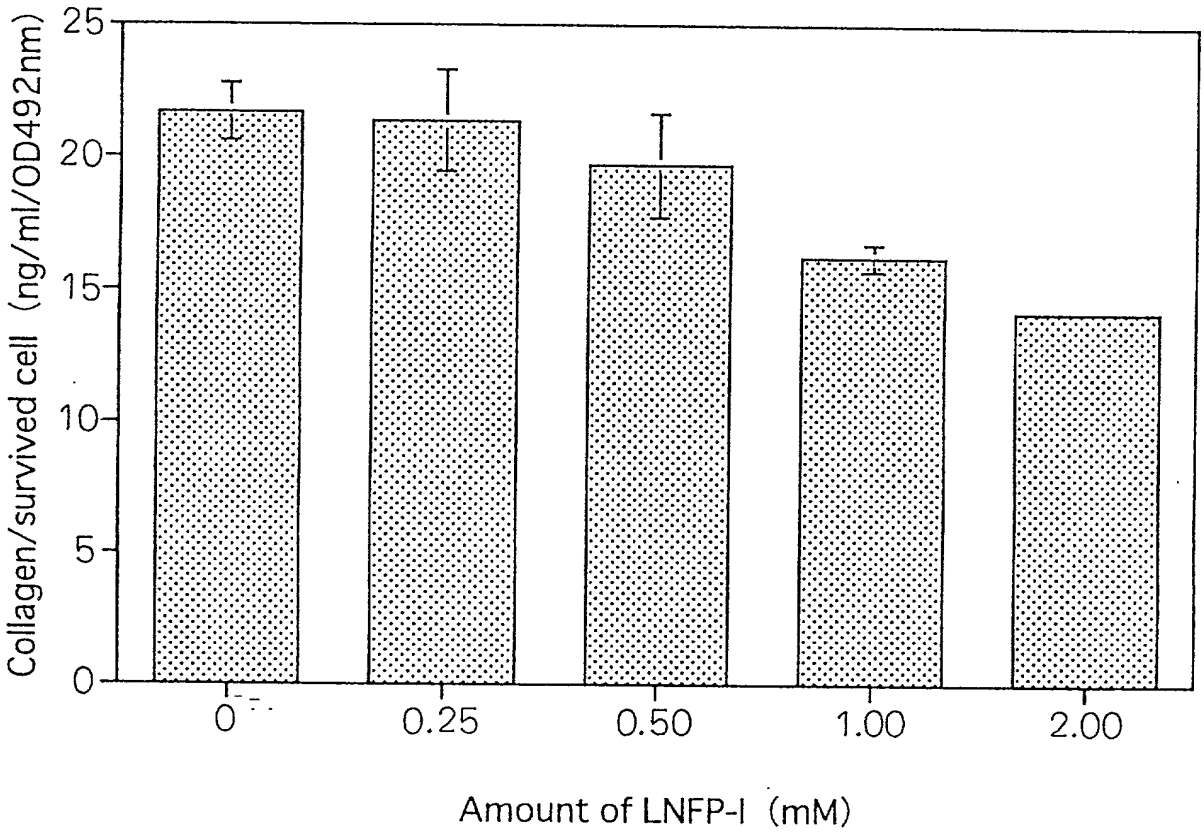


Fig. 6



Declaration and Power of Attorney for Patent Application

特許出願宣言書及び委任状

Japanese Language Declaration

日本語宣言書

下記の氏名の発明者として、私は以下の通り宣言します。

As a below named inventor, I hereby declare that:

私の住所、私書箱、国籍は下記の私の氏名の後に記載された通りです。

My residence, post office address and citizenship are as stated next to my name.

下記の名称の発明に関して請求範囲に記載され、特許出願している発明内容について、私が最初かつ唯一の発明者（下記の氏名が一つの場合）もしくは最初かつ共同発明者であると（下記の名称が複数の場合）信じています。

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

PHARMACEUTICAL COMPOSITION HAVING INHIBITORY EFFECT ON OVERPRODUCTION AND ACCUMULATION OF EXTRACELLULAR MATRIX

上記発明の明細書（下記の欄でx印がついていない場合は、本書に添付）は、

the specification of which is attached hereto unless the following box is checked:

☐ __月__日に提出され、米国出願番号または特許協定条約国際出願番号を_____とし、
（該当する場合）_____ に訂正されました。

☐ was filed on August 5, 1999
as United States Application Number or PCT International Application Number PCT/JP99/04238
and was amended on August 11, 2000
[B2] _____ (if applicable) under PCT Article 34

私は、特許請求範囲を含む上記訂正後の明細書を検討し、内容を理解していることをここに表明します。

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

私は、連邦規則法典第３７編第１条５６項に定義されるとおり、特許資格の有無について重要な情報を開示する義務があることを認めます。

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56.

Japanese Language Declaration

日本語宣言書

私は、米国法典第３５編１１９条(a)-(d)項又は３６５条(b)項に基き下記の、米国以外の国の少なくとも一ヵ国を指定している特許協力条約３６５(a)項に基ずく国際出願、又は外国での特許出願もしくは発明者証の出願についての外国優先権をここに主張するとともに、優先権を主張している、本出願の前に出願された特許または発明者証の外国出願を以下に、枠内をマークすることで、示しています。

I hereby claim foreign priority under Title 35, United States Code, § 119(a)-(d) or § 365 (b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed.

Prior foreign application(s)
外国での先行出願

Priority Not Claimed
優先権主張なし

10-233499 (Pat. Appln.)

[C1] (Number) (番号)	[C2] Japan (Country) (国名)	[C3] 6/August/1998 (Day/Month/Year Filed) (出願年月日)	☐
[C5] (Number) (番号)	[C6] (Country) (国名)	[C7] (Day/Month/Year Filed) (出願年月日)	☐
[C9] (Number) (番号)	[C10] (Country) (国名)	[C11] (Day/Month/Year Filed) (出願年月日)	☐

私は、第３５編米国法典１１９条(e)項に基いて下記の米国特許出願規定に記載された権利をここに主張いたします。

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below.

[D1] (Application No.) (出願番号)	[D2] (Filing Date) (出願日)	[D3] (Application No.) (出願番号)	[D4] (Filing Date) (出願日)

私は、下記の米国法典第３５編１２０条に基いて下記の米国特許出願に記載された権利、又は米国を指定している特許協力条約３６５条(c)に基ずく権利をここに主張します。また、本出願の各請求範囲の内容が米国法典第３５編１１２条第１項又は特許協力条約で規定された方法で先行する米国特許出願に開示されていない限り、その先行米国出願書提出日以降で本出願書の日本国内または特許協力条約国際提出日までの期間中に入手された、連邦規則法典第３７編１条５６項で定義された特許資格の有無に関する重要な情報について開示義務があることを認識しています。

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

[E1] (Application No.) (出願番号)	[E2] (Filing Date) (出願日)	[E3] (Status)(patented, pending, abandoned) (現況：特許許可済、係属中、放棄済)
[E4] (Application No.) (出願番号)	[E5] (Filing Date) (出願日)	[E6] (Status)(patented, pending, abandoned) (現況：特許許可済、係属中、放棄済)

私は、私自身の知識に基ずいて本宣言書中で私が行なう表明が真実であり、かつ私の入手した情報と私の信じるところに基ずく表明が全て真実であると信じていること、さらに故意になされた虚偽の表明及びそれと同等の行為は米国法典第１８編第１００１条に基ずき、罰金または拘禁、もしくはその両方により処罰されること、そしてそのような故意による虚偽の声明を行なえば、出願した、又は既に許可された特許の有効性が失われることを認識し、よってここに上記のごとく宣誓を致します。

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Japanese Language Declaration

日本語宣言書

委任状： 私は下記の発明者として、本出願に関する一切の手続きを米特許商標局に対して遂行する弁理士または代理人として、下記の者を指名いたします。（弁護士、または代理人の氏名及び登録番号を明記のこと）

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith: *(list name and registration number)*

John H. Mion, Reg. No. 18,879; Thomas J. Macpeak, Reg. No. 19,292; Robert J. Seas, Jr., Reg. No. 21,092; Darryl Mexic, Reg. No. 23,063; Robert V. Sloan, Reg. No. 22,775; Peter D. Olexy, Reg. No. 24,513; J. Frank Osha, Reg. No. 24,625; Waddell A. Biggart, Reg. No. 24,861; Louis Gubinsky, Reg. No. 24,835; Neil B. Siegel, Reg. No. 25,200; David J. Cushing, Reg. No. 28,703; John R. Inge, Reg. No. 26,916; Joseph J. Ruch, Jr., Reg. No. 26,577; Sheldon I. Landsman, Reg. No. 25,430; Richard C. Turner, Reg. No. 29,710; Howard L. Bernstein, Reg. No. 25,665; Alan J. Kasper, Reg. No. 25,426; Kenneth J. Burchfiel, Reg. No. 31,333; Gordon Kit, Reg. No. 30,764; Susan J. Mack, Reg. No. 30,951; Frank L. Bernstein, Reg. No. 31,484; Mark Boland, Reg. No. 32,197; William H. Mandir, Reg. No. 32,156; Brian W. Hannon, Reg. No. 32,778; Abraham J. Rosner, Reg. No. 33,276; Bruce E. Kramer, Reg. No. 33,725; Paul F. Neils, Reg. No. 33,102; Brett S. Sylvester, Reg. No. 32,765; Robert M. Masters, Reg. No. 35,603, George F. Lehnigk, Reg. No. 36,359, John T. Callahan, Reg. No. 32,607 and Steven M. Gruskin, Reg. No. 36,818.

32

書類送付先

Send Correspondence to:
SUGHRUE, MION, ZINN, MACPEAK & SEAS, PLLC
2100 Pennsylvania Avenue, N.W. Suite 800
Washington, D.C. 20037-3213

直接電話連絡先： （名前及び電話番号）

Direct Telephone Calls to:

(202)293-7060

唯一または第一発明者名 1-00	Full name of sole or first inventor Satoshi Sasaki
発明者の署名 日付	Inventor's signature Date December 28, 2000
住所	Residence Hino-shi, Tokyo, Japan JPX
国籍	Citizenship Japanese
私書箱	Post Office Address c/o TEIJIN LIMITED Tokyo Research Center, 3-2, Asahigaoka 4-chome, Hino-shi, Tokyo 191-0065, Japan
第二共同発明者 2-00	Full name of second joint inventor, if any Yoshihiko Sumi
第二共同発明者 日付	Second inventor's signature Date December 28, 2000
住所	Residence Hino-shi, Tokyo, Japan JPX
国籍	Citizenship Japanese
私書箱	Post Office Address c/o TEIJIN LIMITED Tokyo Research Center, 3-2, Asahigaoka 4-chome, Hino-shi, Tokyo 191-0065, Japan

（第三以降の共同発明者についても同様に記載し、署名をすること）

(Supply similar information and signature for third and subsequent joint inventors.)

over

Japanese Language Declaration

日本語宣言書

第三の共同発明者の氏名（該当する場合）	3-00	Full name of third joint inventor, if any Reginald Colin Hughes
同第三発明者の署名　日付		Third inventor's signature　Date R Colin Hughes December 28, 2000
住所		Residence London, Greater London, United Kingdom GBX
国籍		Citizenship British
郵便の宛先		Post office address National Institute for Medical Research, The Ridgeway, Mill Hill,
		London, Greater London NW7 1AA United Kingdom
第四の共同発明者の氏名（該当する場合）		Full name of fourth joint inventor, if any
同第四発明者の署名　日付		Fourth inventor's signature　Date
住所		Residence
国籍		Citizenship
郵便の宛先		Post office address
第五の共同発明者の氏名（該当する場合）		Full name of fifth joint inventor, if any
同第五発明者の署名　日付		Fifth inventor's signature　Date
住所		Residence
国籍		Citizenship
郵便の宛先		Post office address
第六の共同発明者の氏名（該当する場合）		Full name of sixth joint inventor, if any
同第六発明者の署名　日付		Sixth inventor's signature　Date
住所		Residence
国籍		Citizenship
郵便の宛先		Post office address